Exocytosis of Residual Bodies in a Lysosomal Storage Disease

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Scanning and transmission electron-microscopic study of the livers of 2 kittens suffering from G_{M2} gangliosidosis revealed discontinuities in the plasma membrane of hepatocytes and Kupffer cells, indicating an attempt by the cells to free themselves of excess lysosomal residues by means of exocytosis. Inclusions morphologically similar to those seen in the hepatocytes were observed in the perisinusoidal space. Openings were seen on all surfaces of the hepatocytes. Such extrusion of lysosomal residues is an example of a mechanism rarely observed in viable metazoan cells. (Am J Pathol 98:385-394, 1980)

LYSOSOMAL STORAGE diseases are characterized by an intracellular accumulation of products resulting from the incomplete digestion of a substrate by lysosomal enzymes. The incomplete digestion resulting from a deficit of one or more lysosomal enzymes leads to the accumulation of a product that is unable to diffuse through the limiting lysosomal membrane. The continued buildup of such substances, specific for the disease, often becomes so extensive as to interfere with the normal functional capacity of the cell.

Although exocytosis, or cellular defecation, is a common means by which protozoa rid themselves of residual bodies resulting from lysosomal digestion, evidence for such a mechanism operating in metazoan cells is equivocal. Reports of such occurrences in the kidney [1] and in the liver [2] have been based on the observation of residual bodies in the lumen of the proximal tubule and in the bile canaliculus, respectively. Critics have noted that the observed location of these bodies could be explained as being due to the action of mechanical forces during tissue collection.[3] Others have observed remnants of hepatic residual bodies extracellularly following the stress of hypoxia [4,5] or toxic drugs;[6] but cell death, rather than exocytosis, has been suggested as a mechanism.[7] Evidence derived from lysosomal activity following their being loaded with various markers—Thoratrast,[8] ferritin,[9] colloidal gold [10]—appears circumstantial, be-

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cause in no case were images of stages of exocytosis recorded. Similar evidence for exocytosis of lysosomal residues into bile canaliculi of the newborn has been reported.[11]

G_{M2} gangliosidosis, a lysosomal storage disease resulting from deficits of isoenzymes of hexosaminidase, has recently been reported in cats.[12,13] A feature of this disease is accumulation of globoside within lysosomal membranes in the hepatocyte. Study of the livers of two kittens affected by this disease gave evidence that following the extreme buildup of globoside in the hepatocyte there was extrusion of the included material from the cell by exocytosis.

Materials and Methods

The kittens were anesthesized and, after heparinization, were perfused via the left ventricle with 4% paraformaldehyde in phosphate buffer in one case and Karnovsky's solution in the other. Perfusion pressure was maintained at 160–180 mm Hg. For transmission electron microscopy (TEM), tissue blocks were rinsed overnight in buffer, further fixed in 2% OsO_4 in the same buffer, dehydrated, and embedded in a mixture of Dow epoxy resins (DER 732 and 332) or in Spurr's medium. Sections were cut with diamond knives, stained with uranyl acetate and lead citrate, and examined in a RCA EMU-4A electron microscope.

Blocks for scanning electron microscopy (SEM) study were dehydrated and cryofractured in ethanol,[14] critical-point–dried, and sputter-coated with gold or gold–palladium.

Other blocks were fractured manually [15] either before or after critical-point–drying. Tissue from normal, unrelated kittens of comparable age was processed similarly for use as control specimens. Prepared blocks were examined in either a Kent Cambridge Mark II A or a JEOL JSM-35 scanning electron microscope.

Results

Hepatocytes, Kupffer cells, and sinusoidal endothelial cells of affected kittens contained numerous membrane-bound pleomorphic inclusions (Figure 1). Some were small and discrete. Fusion of the limiting membranes of these discrete structures appeared to result in the formation of large vacuoles containing numerous inclusions, most of which appeared as membranous whorls in transmission electron micrographs.

The observation of similar membranous bodies in the perisinusoidal space (Figure 2) prompted a search that revealed instances of fusion of the limiting membrane of a cytoplasmic vacuole with the hepatocyte plasma membrane, placing the lumen of the vacuole in continuity with the perisinusoidal space, and allowing for release of the contained material (Figure 3). This same appearance was occasionally encountered in cryofractured liver (Figure 4). This technique also revealed evidence of fusion of adjacent vacuoles and for the globular shape of the included material (Figure 5). Globular material similar to that seen in fractured vacuoles was often observed in the sinusoidal lumen or in the perisinusoidal space,

and "pointing" of cone-shaped extensions of superficial cytoplasmic vacuoles toward the hepatocyte plasma membrane was common.

In manually fractured liver, numerous openings or holes were seen on all surfaces of the hepatocytes (Figures 6, 7, and 8). Most had diameters between 0.5 and 2.0 μ with very few larger (Figures 6 and 7) or smaller (Figure 8). No clear examples of openings into bile canaliculi were noted, but inclusions were seen in canaliculi by transmission electron microscopy.

Surface openings were less numerous in the hepatocytes of the less severely affected kitten, and only those with diameters smaller than 0.5 μ were observed in control livers. Similar discontinuities in the surface membrane of Kupffer cells were occasionally seen in fractured preparations (Figure 6) but not by TEM. Their incidence was lower than that in the hepatocytes.

Discussion

The membranous or globular material seen within the hepatocytes when viewed by means of TEM or SEM is presumed to be globoside, which is found in abnormally high concentrations in the livers of affected kittens.[12] It is further presumed that the vacuoles containing these inclusions are secondary lysosomes.[16] Electron-microscopic examination of affected liver tissue prepared by a variety of methods provided evidence of an attempt on the part of the hepatocytes and the Kupffer cells to rid themselves of this excess accumulation of globoside by means of exocytosis. That this occurs extensively throughout the liver was evidenced by the appearance of openings in hepatocyte surfaces in nearly every field examined in manually fractured liver. Despite the ease of their demonstration by this technique, their distribution was sparse enough to make them very difficult to encounter on thin sections by TEM examination.

Similar "holes" have been observed by others [17] in normal livers. These were small in diameter, less than 0.5 μ, and similar to the small-diameter openings we observed in both affected and control tissues. These have been postulated to be pinocytotic caveolae or sites of release of very low density lipoproteins. It is very possible that some of these small openings observed in affected hepatocytes represent the initial vacuolar openings, which would subsequently have become enlarged.

The appearance of these openings on all surfaces of the cells implies that the process of extrusion is nondirectional and can be assumed to be passive and fortuitous; the fusion of the membrane of a vacuole with the plasma membrane being a process similar to the fusion of the membranes of two cytoplasmic vacuoles resulting in their coalescence. The lesser oc-

currence of exocytosis in the less advanced case indicates that a certain level of accumulation within the cell may first be necessary. However, the need for further cytochemical study of the lysosomal membranes is suggested in order to determine whether differences do exist between the membranes that fuse with the plasma membrane and those that do not. Information concerning such differences, if they should be found to exist, could be valuable in devising therapeutic techniques that might result in the "unloading" of affected cells.

The fate of the released product is unknown. On morphologic grounds, it can be assumed that most reaches the perisinusoidal space, even that released on the lateral surfaces. From the perisinusoidal space it may enter the blood or be carried to the lymphatics of the portal areas. This mechanism may, at least in part, explain circulating levels of abnormal components in various of the storage diseases. The ability to view this phenomenon in the animal model by means of the techniques described herein will allow for monitoring and evaluation of methods aimed at causing cells to dump their stores of lysosomal residues into the circulation, where they may be more accessible to exogenous enzymes.

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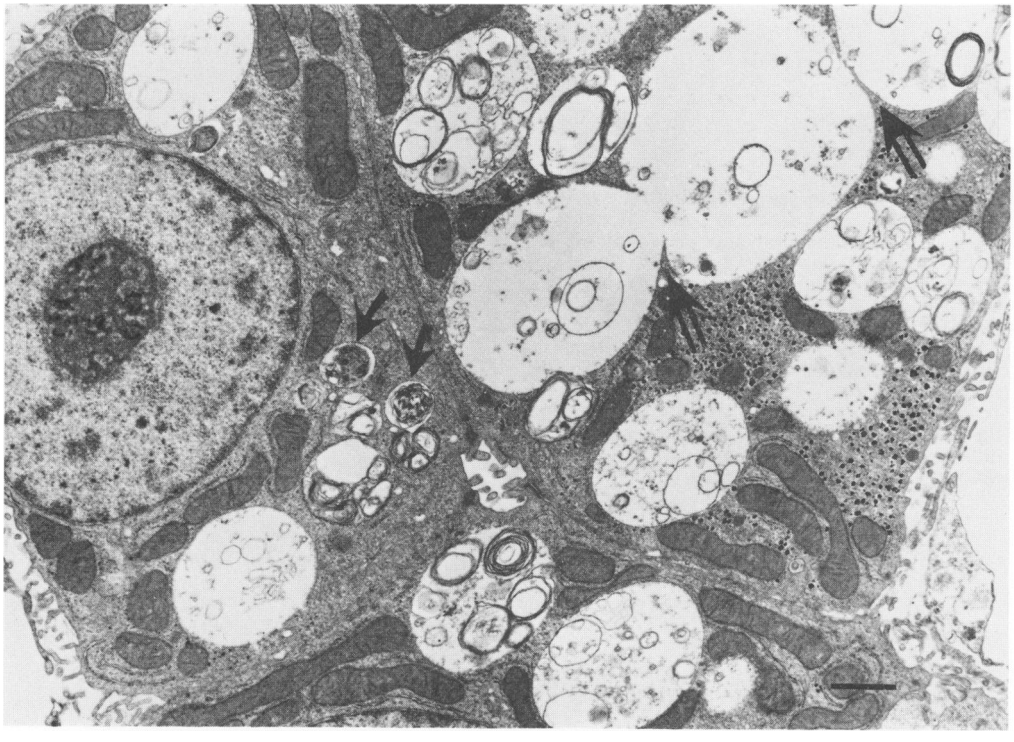
Figure 1—Hepatocytes with cytoplasmic vacuoles of varying size from affected kitten. Multiple membranous inclusions are seen in the larger vacuoles, but some smaller vacuoles contain only one such body (*arrows*). Fusion of adjacent vascuoles is suggested (*double arrows*). Marker equals 1 μ. (×8800)

Figure 2—Perisinusoidal space flanked by hepatocyte (*H*) and endothelial cell (*E*) containing membranous bodies morphologically similar to those seen in hepatocyte vacuoles. Marker equal 1 μ. (×17,700)

Figure 3—Peripheral cytoplasmic vacuole opening into perisinusoidal space. Marker equals 1 μ. (×17,000)



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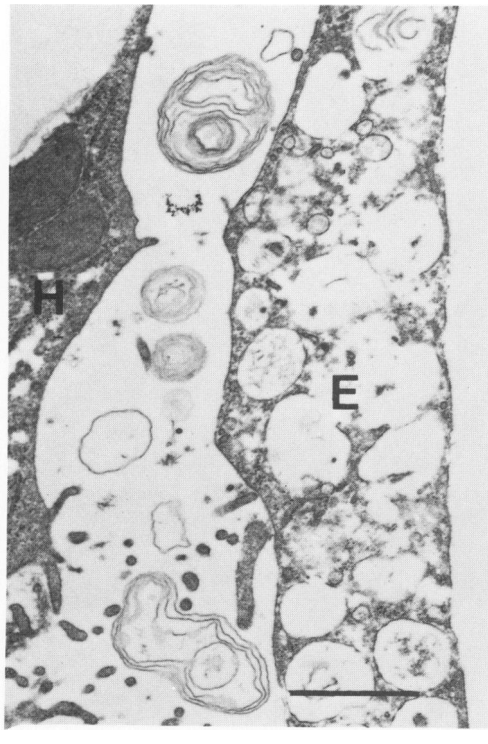

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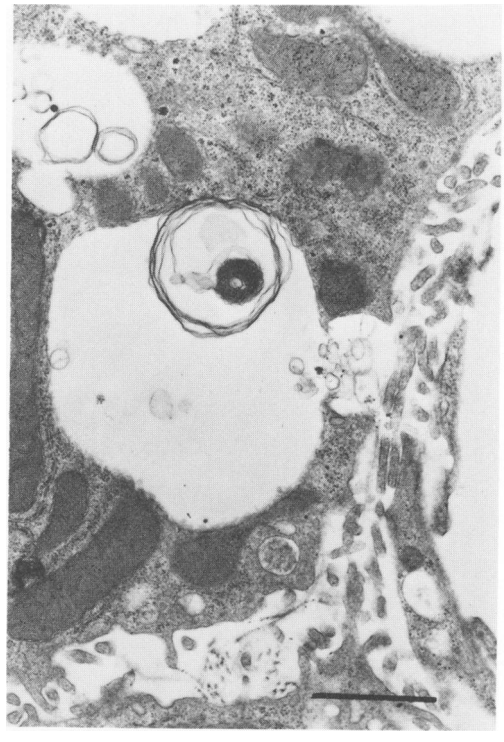

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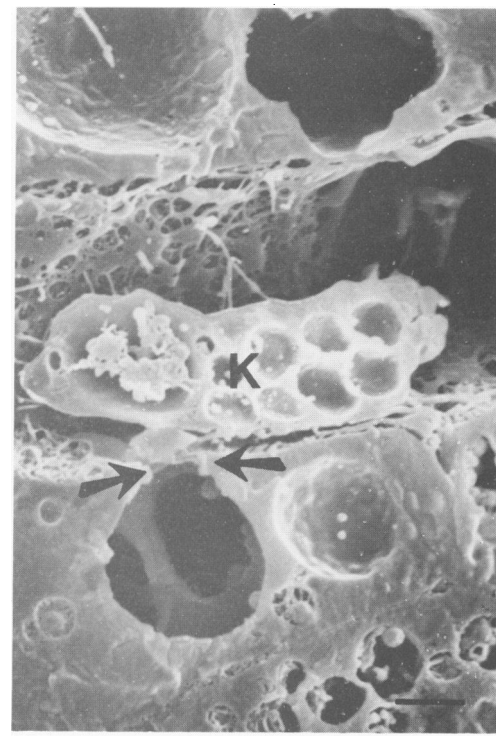

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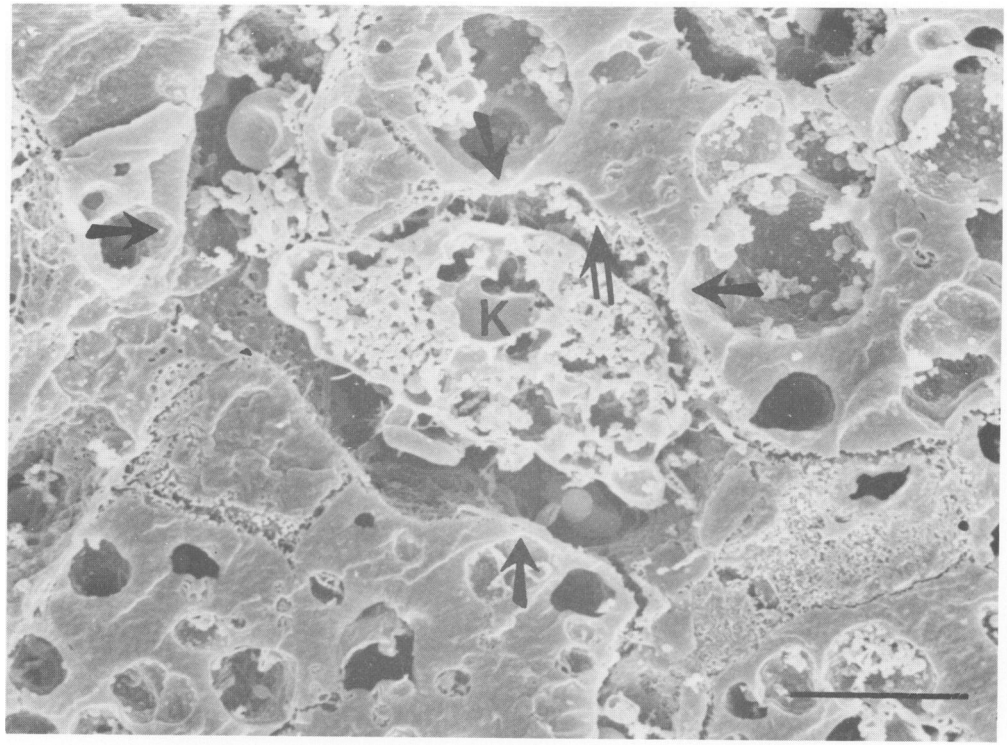
Figure 4—Scanning electron micrograph of cryofractured liver, illustrating the continuity of the lumen of a cytoplasmic vacuole with the perisinusoidal space (*arrow*). Openings in the wall of the vacuole represent sites of fusion with other vacuoles. A fractured Kupffer cell (*K*) lies in the sinusoid. Marker equals 2 μ. (×4300) **Figure 5**—Survey of cryofractured liver shows numerous superficial cytoplasmic vacuoles with asymmetric outlines "pointing" toward the cell surface (*arrows*). The globular shape of the included material is evident in the vacuoles, and some can be noted in the perisinusoidal space (*double arrow*). Marker equals 10 μ. (×2400)



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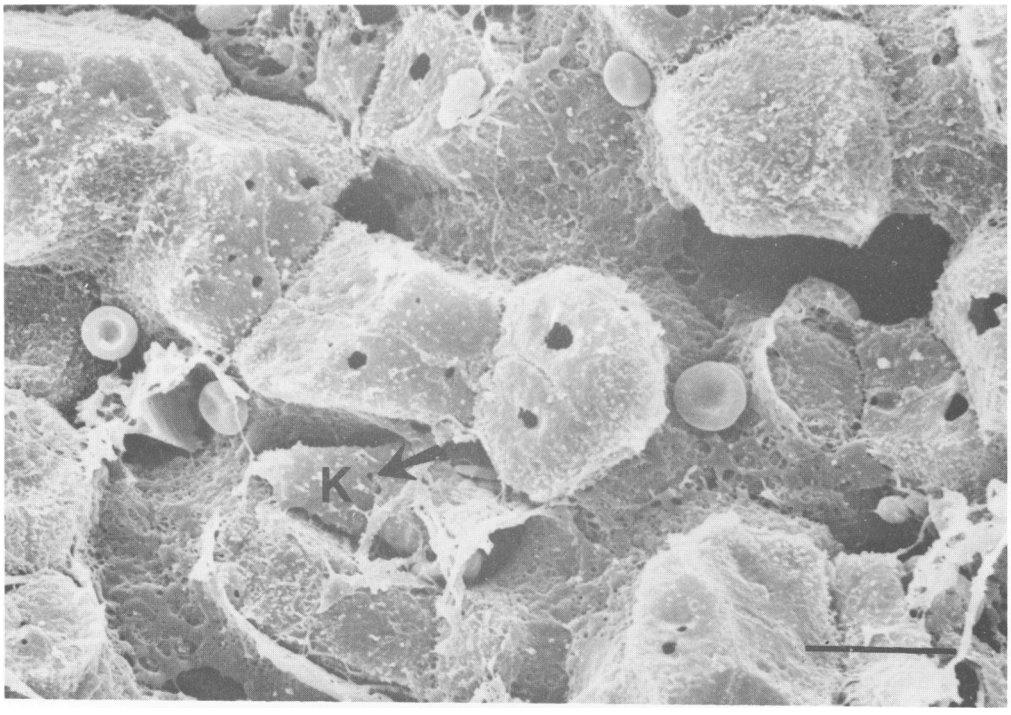

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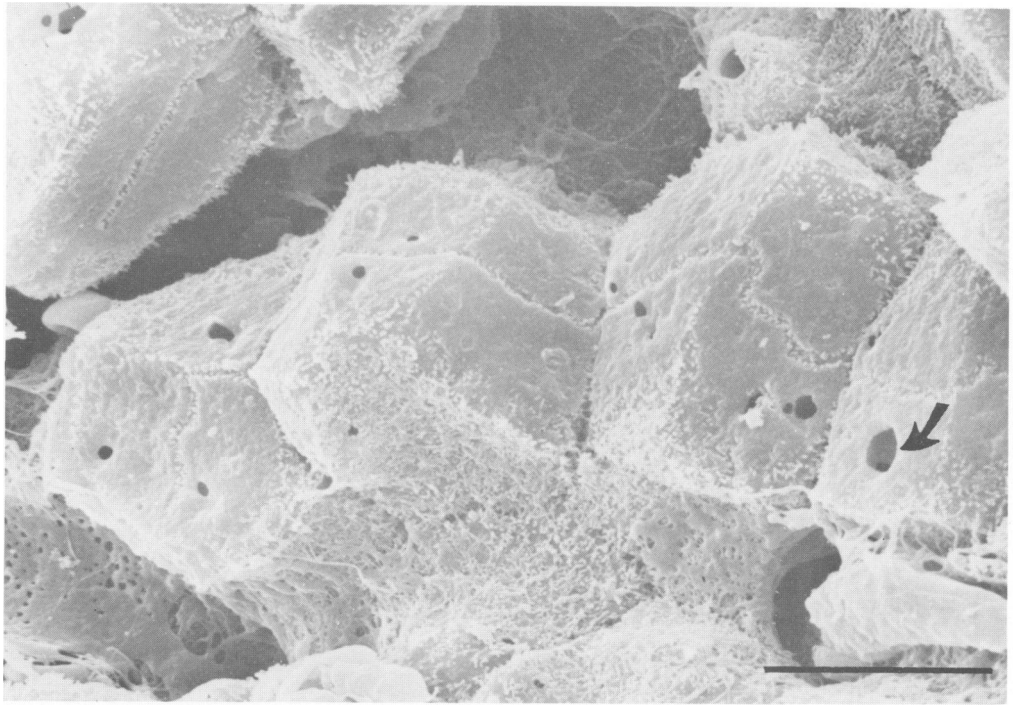

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Figure 6—Survey of manually fractured liver showing typical distribution of openings or holes in hepatocyte plasma membranes. A Kupffer cell (*K*) also has holes (*arrow*). Marker equals 10 μ. (×2000) **Figure 7**—Higher magnification of hepatocytes with discontinuities on all surfaces. Note that variation in size is within a rather restricted range. Opening at right (*arrow*) reveals second opening, probably representing the site of fusion of two vacuoles. Marker equals 10 μ. (×3000)

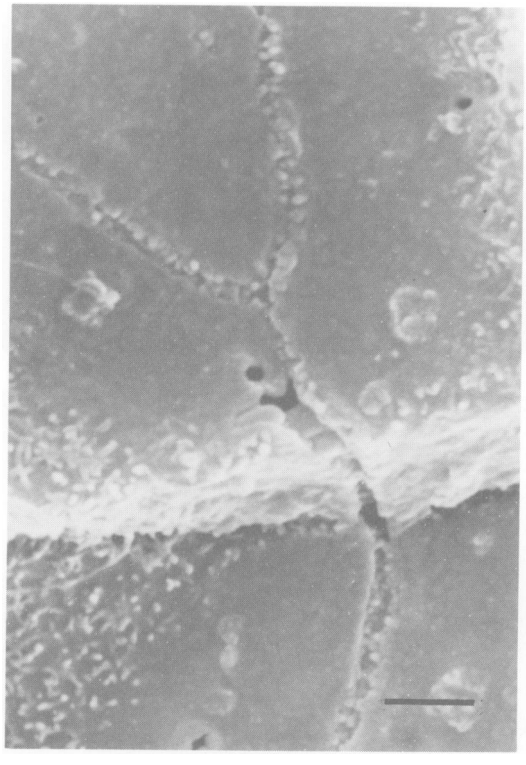

Figure 8—Small discontinuities (0.5 μ) as observed in affected hepatocytes (as here) and in control tissue. Most commonly seen on smooth, apposed surfaces. Marker equals 2 μ. (×5500)